

Application No.: 10/811,423
Response dated: February 16, 2007
Reply to Office Action dated: October 16, 2006

Amendments to the Specification:

Please replace paragraph [00016] and [00028] with the following amended paragraph:

[00016] This investigation was to evaluate the effects of O₂ and CO₂ atmosphere on the ability of human embryonic stem cells to proliferate and remain undifferentiated. Cells were cultured in modified DMEM/DF12 plus ~~"Knock-out"~~ "KNOCK-OUT" (Trademark) Serum Replacement from Gibco. The "KNOCK-OUT" (Trademark) Serum Replacement medium generically contains albumin, amino acids, vitamins, minerals, at least one transferrin or transferrin substitute, and at least one insulin or insulin substitute. The modified media included an adjustment to the sodium bicarbonate and sodium chloride concentrations to moderate pH and osmolarity. All media were corrected for appropriate pH given the concentration of CO₂, and the osmolarity was adjusted to also remain constant. Ultimate pH and osmolarity were constant across all media tested, despite alteration of atmospheric conditions. All media were conditioned overnight at standard atmospheric conditions (5% CO₂ in air) on mouse embryonic fibroblasts (MEFs) plated at a density of 2.12×10^5 cells/ml prior to experimental use. Conditioning of the medium for stem cell culture is done to induce the stem cells to remain undifferentiated without exposing the cells to the MEFs themselves. Conditioning means the medium is used to culture MEFs before the medium is used to culture stem cells. Although the MEFs are removed from the conditioned medium prior to introduction of the stem cells, the medium is conditioned in some poorly understood manner and supports culture of undifferentiated stem cells in a manner that unconditioned media do not.

[00028] This investigation was to evaluate the effects of osmolarity on the ability of human embryonic stem cells to proliferate and remain undifferentiated. Human stem cells were again cultured in modified DMEM/DF12 plus ~~"Knock-out"~~ "KNOCK-OUT" (trademark) Serum Replacement from Gibco (as described above). The osmolarity was adjusted to the levels set forth below by adjustment of the amount of sodium chloride in each medium. Media were all tested to assure that no alterations in pH or buffering capacity

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resulted from the adjustments in osmolarity. All media were again conditioned overnight on MEFs plated at a density of 2.12×10^5 cells/ml prior to experimental use.